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RESEARCH ARTICLE

Influence of Gibberellic acid and Methionine on growth, flowering quality, leaf anatomical structure and genetic diversity of *Chrysanthemum morifolium* Ramat plant

Eman Zaky Othman[1], Rasha Ahmed El-Ziat[1], Hend Mohamed Farag[2]*, Iman Mohamed El-Sayed[3]

[1]Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt, [2]Department of Agriculture Botany, Faculty of Agriculture, Cairo University, Giza 12613, Egypt, [3]Department of Ornamental Plants and Woody Trees, National Research Centre (NRC), Giza 12622, Egypt; imanelsayed6065@yahoo.com

ABSTRACT

Ornamental plants are grown all over the world. In most countries, Chrysanthemum is considered one of the most popular cuts and potted flowers. Plants were sprayed with gibberellic acid (GA_3) at concentrations (100, 200, and 300 ppm) and methionine (ME) at levels (200, 400, and 600 ppm), as bio-growth stimulants in the pre-blooming stage. The morphological and flowering parameters significantly increased by increasing the concentration of GA_3. The highest values of K%, P%, total carbohydrates content, total phenols, total chlorophylls, and carotenoids in the leaf were obtained from gibberellic acid treatment at the rate of 300 ppm, while methionine at 600 ppm gave the highest value of protein in flowers. Also, various levels of gibberellic acid application significantly showed variation for days to initiation of Chrysanthemum flowers. The results of leaf anatomy showed an increase in most characteristics such as (thickness of the main vein, lamina, and spongy tissue) under study when spraying with gibberellic acid, especially at the concentration of 300 and methionine 600 ppm; respectively compared with control. Furthermore, inter-simple sequence repeat (ISSR) analysis has provided a powerful molecular marker for identifying variation with control and the best treatments. Therefore, the utilization of 300 ppm GA_3 a plant growth regulator and 600 ppm ME an amino acid, these treatments are recommended to enhance Chrysanthemum parameters which lead to increasing its economic value as cut flowers and flowering potted plants as well as pharmaceuticals industries and multi-chemical uses.

Keywords: Cut flowers; Bio-growth stimulants; ISSR; Leaf anatomy, Growth regulators.

INTRODUCTION

Flower growing is spread worldwide and produces a positive economic income. The ornamental section includes cut green flowers, bedding flowers, and potted indoor plants. (El-Sayed et al., 2020 and Soliman et al., 2022, Soliman and El-Sayed, 2023).*Chrysanthemum morifolium* is a popular and economically crucial floricultural crop in the family Asteraceae. chrysanthemum blooms come in a variety of colors, forms, and sizes (El-Sayed and El-Ziat, 2021). As the king of flowers, the chrysanthemum is one of the world's most important cut flowers, pot plants, and medicinal plants (Liu et al., 2021).Chrysanthemum cut flowers are one of the most essential flower arrangements due to their long vase life and having beautiful, appealing, charming flowers. It can be planted in the garden chrysanthemum is flowers that possess medicinal property. Chrysanthemum is native to the northern hemisphere, specifically Europe and Asia. It is a perennial herbaceous plant with a fibrous root system sensitive to water longing and reaches a height of 50-150 cm; The United States National chrysanthemum society divides chrysanthemum flowers into thirteen different bloom forms. Terminal cuttings are used to propagate chrysanthemum commercially for short-day flowering that are blooming in the late autumn (Taghipour et al., 2021).

Plant growth regulators are natural elicitors vital for implant development, improving seed quality and yield (Sumi et al., 2021Khalil et al., 2022). Gibberellic acid is a plant growth regulator that enhances seed germination, stem elongation, growth, photosynthesis, cell expansion, and flowering due to its phytohormones roles (Mzabri et al., 2021, Hassan et al., 2023). Previous studies reported that

***Corresponding author:**
Hend Mohamed Farag, Department of Agriculture Botany, Faculty of Agriculture, Cairo University, Giza 12613, Egypt.
E-mail: hendfaragagr@gmail.com

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gibberellic acid can stimulate elongation, leaf area index, plant dry mass, growth, photosynthesis, flowering, nutrient transport, and yield of mustard, and improve the quality and productivity of several ornamental plants and crops (Shah et al., 2006; Kirmizi et al., 2017). Furthermore, GA_3 application also increases the number of flowers and initiates early flowering in several ornamental plants, in the case of the *Chrysanthemum* plant (Kumar et al., 2003,and Xylia et al., 2022).

Amino acids have several vital roles in a plant's central metabolism. Essential amino acids (EAAs), such as methionine and lysine, cannot be produced by humans or animals and must thus be obtained through food (Galili, et al., 2016, and Yang, et al., 2020). Amino acids also regulate many metabolic, other biochemical, and physiological pathways, affecting a wide range of physiological activities in plants (Amir, et al., 2018and Yang, et al., 2018). In addition, amino acids are essential as hormone precursors, stress-relieving mediators, and nitrogen sources (Maeda and Dudareva, 2012,and Albadwawi et al., 2022).Although amino acids usually are present in the soil, the plant cannot absorb them without transport to their roots (Amir, et al., 2019and Khan, et al., 2020). Methionine is a sulfur-containing essential amino acid found in seeds and plant tissues (Krishnan and Jez, 2018). Its quantity affects the nutritional value of crops because it is comparable to the base for animal and human proteinaceous food (Amir and Ma 2012, Krishnan and Jez, 2018). Furthermore, methionine performs an essential role in plant cells. It acts as a building block for protein synthesis, with its codon serving as the starting point for protein mRNA translation. Indirectly, methionine affects various cellular activities through its primary catabolic product, S- a demosyl methionine (SAM). SAM is the precursor for ethylene synthesis (Roje, 2006; Yang and Hoffman, 1984). Polyamines have a crucial function in cell proliferation and differentiation and plant growth apoptosis, homeostasis, and gene expression in plants (Pang, et al., 2007). Therefore, owing to the significant role of gibberellic acid and methionine in improving plant growth, yield, and chemical composition, *C.morifolium* cv. "Zambla Yellow" is an economical and important plant subjected to applying GA_3 and ME to understand the interaction between GA_3 and ME and *Chrysanthemum*. To the best of our knowledge, reports on the application of methionine of *C.morifolium* Ramat Zambla Yellow" are limited.

In the previous studies, different concentrations of gibberellic acid (GA3) and methionine (ME) were applied to enhance the morphology and flowering in various plants such as in *Ammimajus* L. found that 100 mg/l of GA_3 gave a significant effect on growth characteristics (Uddinet al.,2023). In addition, Aziyituni,(2023) revealed that using gibberellic acid (GA_3) at 150 mg/l gave the maximum values of morphological and flowering parameters as well as a chemical composition than control on *Tagetserecta*. Furthermore, Mehak et al., (2021) showed that foliar applications of sunflower plants with methionine enhance growth and flowering traits. Rezaeian et al., (2022) reported that applying methionine 200 mg/l to eggplant increases the flowering yield and late flowering genotype. Moreover, EL-Bauome et al.,(2022) demonstrated that treatment plants with methionine increased the growth of cauliflower.

Therefore, the current research aimed to examine the impact of different concentrations of gibberellic acid and methionine on flowers' production, chemical composition, leaf anatomical structure, and detection of genetic variation of *Chrysanthemum morifolium*, Ramat "Zambla yellow".

MATERIALS AND METHODS

Plant materials and growth conditions

The current experiment was performed during two successive seasons of 2021 & 2022, at the Ornamental Horticulture Department, in an open greenhouse at the Faculty of Agriculture, Cairo University, Giza, Egypt. *Chrysanthemum morifolium* Ramat "Zambla yellow" seedlings were attained from the commercial farm, in Giza, Egypt. The seedlings were planted with lengths of 8-9 cm and four to six leaves/seedling in plastic pots (30 cm). Two seedlings were planted in each pot, with soil comprised clay with sand at 1:1 v/v. Soil analysis was performed at the soil testing laboratory, Faculty of Agriculture, Cairo University, Giza, Egypt in (Table 1).

Experiments treatments

Gibberellic acid (GA_3) treatments in this study were at three concentrations (100, 200, and 300 ppm), and Methionine (ME) was at three levels (200, 400, and 600 ppm) as a foliar spray application. The plants were sprayed three times in the morning until they reached the running-off point. The first spray after planting for 21 days. After that, two applications were sprayed at three-week intervals, using "Tween 20" at 1 ml/L as a surfactant substance to reduce the water tension and improve the treatment efficiency.

Morphological parameters

For both evaluation seasons, plant height (cm), the number of branches/plant, the number of leaves/plant and total leaf area (cm^2/plant) (leaf area were measured using the following formula [23](Leaf area (cm^2)= leaf dry weight (g)× disk area (cm^2/disk)/dry weight (g)), stem diameter

Table 1: Initial soil physical and chemical analysisof the experimental site before treatment application

The physical and chemical analysis of the experimental soil	
Organic matter (%)	2.1
$CaCO_3$%	12.4
Clay%	17.97
Silt %	11.80
Sand%	70.23
Texture class	sandy loam
pH (Extract 1/2.5 H_2O)	7.8
ECE 24°C (ds/m)	3.3
Soluble ions (meq/L)	
HCO^{-3}	2.6
Cl^-	9.8
Fe	1.7
Ca^{+2}	7.2
Mg^{+2}	2.11
Na^+	5.12
Zn	0.39
Mn	0.61
Total N %	0.052
Available *P* ppm	6.74
Exchange K mg/1000g soil	1.51

(mm/plant), fresh weight of shoot (FW) (g/plant) and dry weight of shoot (DW) (g/plant) were determined.

Flowering parameters

The number of flowers per plant, the diameter of head flowers (cm/plant), time showing flower color (days), length of flower stalk (cm/plant), fresh weight (FW), and dry weight (DW) (g) of individual flower/plant.

Chemical analysis

Estimation of pigment content

Extract the crushed leaf samples (0.2g) fresh weight (F.W.) with 85% methanol. The extracts were centrifuged for ten min. at 8000 rpm. UV–Visible spectrophotometer (UV-1280, Shimadzu, Japan) was used to determine the photosynthetic pigments. The equations and specific absorption in the wavelength were 660, 640, and 440 nm to measure chlorophyll a and band carotenoids, respectively as described by (Saric et al, 1967).

Determination of total carbohydrate in leaves and flowers

The concentration of total carbohydrates was prepared in the leaves and flowers using the method described by (Dubois et al., 1956).

Estimation of total phenols% in leaves and flowers

According to previously described by (Swain and Hillis 1959),total phenol in fresh leaves were assessed by colorimetric method, employing a Folin-Ciocateu regent, determined by a spectrophotometer at 650 nm, and using Gallic acid as the standard.

Measurement of nutrients in tissues

Nitrogen measurement (%): Extract for the determination of nitrogen content was obtained from dried herb. Nitrogen content was determined by the modified Kjeldahl method (Cottenie et al., 1982). Protein content was calculated by multiplying N% by 6.25 (Mariotti et al., 2008).Phosphorus measurement (%):Phosphorus content was measured in dried shoot using the ammonium molybdate method (Snell and Snell, 1959). Potassium measurement (%): According to (Snell and Snell, 1959). the potassium content in the digested solution was measured using a flame photometer I dry weight (DW) of the shoot.

Anatomical structure

Leaf samples were collected from plants treated with different concentrations of GA_3 (100, 200 and 300 ppm) and ME (200, 400 and 600 ppm) throughout the second growing season after the second spray. Specimens were killed and fixed in F.A.A. solution (70% formalin, acetic acid, and ethyl alcohol) for 48 hours. After fixation, samples were washed in 50% ethyl alcohol, dehydrated through butyl alcohol series, and embedded in paraffin wax (melting point, 56-58°C). Leaf cross sections were cut using a Leica Rotary Microtome (20 microns thick). Sections were stained with crystal violet–erythrosine and mounted in Canada balsam according to (Nassar and El-Sahhar, 1998). Cross sections were analyzed microscopically and photomicrograph.

The leaf anatomical parameters (µm): Upper and lower epidermis thickness, main vein thickness, lamina thickness, palisade, and spongy tissue thickness, dimensions of vascular bundle, xylem, and phloem tissue thickness.

Statistical Analysis

Randomized Complete Blocks Design (RCBD) with three replicates was conducted to analyze the data. Determining the differences among averages of studied treatments depended on significance level ($p \leq 0.05$) by using Duncan's multiple range tests (Duncan, 1955). The obtained data were processed by COSTATV-63 software program.

ISSR-PCR analysis

Amplification and analysis of ISSR-PCR for Untreated and mutant *Chrysanthemum* plants, Kit cat no # 69104 (Qiagen Sciences, Maryland, USA) was used to extract genomic DNA from these plants according to the manufacturer's instructions. To detect polymorphism between untreated and treated plants, ten standard ISSR-PCR primers were employed, as described in Table 2. The amplification reaction was performed in 25 µl reaction volume containing 12.5 µl Master Mix (sigma), 2.5 µl primer (10pcmol), 3 µl template DNA (10ng) and 7 µl dH_2O, according to (Ibrahim et al.,2019). PCR amplification was performed

in a Perkin-Elmer/Gene Amp® PCR System 9700 (PE Applied Biosystems) programmed to accomplish 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 45°C for 1 min, and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7min at 72°C in the final cycle. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000). For ISSR analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples and final data sets included both polymorphic and monomorphic bands. Then, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated between genotypes using the unweighted pair group method with arithmetic averages (UPGMA). This matrix was used to construct a phylogenetic tree (dendrogram) was performed according to the Euclidean similarity index using the PAST software Version 1.91 (Hammer et al., 2001).

Table 2: ISSR primers were used in the detection of polymorphism

Primer Name	Sequence
ISSR- 01	5'-AGAGAGAGAGAGAGAGC-3'
ISSR- 02	5'-AGAGAGAGAGAGAGAGG-3'
ISSR- 03	5'-ACACACACACACACACT-3'
ISSR- 04	5'-ACACACACACACACACG-3'
ISSR- 05	5'-GTGTGTGTGTGTGTGTG-3'
ISSR- 06	5'-CGCGATAGATAGATAGATA-3'
ISSR- 07	5'-GACGATAGATAGATAGATA-3'
ISSR- 08	5'-AGACAGACAGACAGACGC-3'
ISSR- 09	5'-GATAGATAGATAGATAGC-3'
ISSR- 10	5'-ACACACACACACACACA-3'

A: Adenine, T: Thymine, G: Guanine, C: Cytosine

RESULTS AND DISCUSSION

Morphological parameters

The Morphological parameters (plant height, number of branches, number of leaves, total leaf area, stem diameter, fresh and dry weights of shoot) were significantly affected by gibberellic acid and methionine Table 3 and Fig.1. The plant height, number of branches, and number of leaves in both seasons were greatly augmented by the foliar application of gibberellic acid. GA_3 foliar application at the rate of 300 ppm increased plant height, the number of branches, the number of leaves, fresh weight of shoot, and dry weight of shoot (74.73,14.34,62.00, 81.24, 22.6 and 71.33, 12.55, 59.00,75.41, 21.86), in the first and second season, respectively. On the other hand, significant reductions of the plant height, number of branches, number of leaves fresh weight of shoot, and dry weight of shoot in both seasons were recorded in the control plants (43.67, 7.30, 35.00, 28.22, 9.31 and 38.33, 6.26, 31.00, 25.11, 7.53 in both seasons, respectively). Moreover, the highest value of total leaf area and stem diameter was obtained from methionine at the rate of 600 ppm compared to the control and other treatments. The following study is in agreement with (Ravat and Nirav, 2015) revealed that using

Table 3: Impact of different concentrations of gibberellic acid (GA_3) and methionine (ME) on the morphological parameters of *Chrysanthemum morifolium* Ramat"Zambla yellow" plant during 2021 and 2022 seasons

Treatments	First season						
	Plant height (cm)	No. of branches/plant	No. of leaves/plant	Total leaf area/ plant (cm^2)	Stem diameter (mm)	F.W shoot (g)/plant	D.W shoot (g)/plant
Control	43.67^{e}	7.30_{c}	35.00^{d}	126.70^{f}	3.69^{c}	28.22^{f}	9.31^{e}
GA_3 100 ppm	57.83^{cd}	10.68^{abc}	54.00^{b}	309.10^{d}	4.71^{bc}	53.11^{d}	15.93^{c}
GA_3 200 ppm	62.66^{bc}	11.86^{ab}	57.00^{ab}	380.30^{bc}	4.84^{b}	60.15^{c}	18.04^{bc}
GA_3 300 ppm	74.73^{a}	14.34^{a}	62.00^{a}	441.10^{ab}	5.32^{ab}	81.24^{a}	22.6^{a}
ME 200 ppm	60.33^{c}	11.08^{abc}	$55.\ 00^{ab}$	$355\ 0.00^{cd}$	5.07^{ab}	63.04^{c}	18.28^{b}
ME 400 ppm	52.17^{d}	9.133^{bc}	44.00^{c}	202.90^{e}	4.57^{bc}	44.09^{e}	13.22^{d}
ME 600 ppm	71.46^{ab}	13.22^{a}	60.00^{ab}	457.70^{a}	5.90^{a}	70.11^{b}	21.03^{a}
	Second season						
	Plant height (cm)	No. of branches/plant	No. of leaves/plant	Total leaf area/ plant (cm^2)	Stem diameter (mm)	F.W shoot (g)/plant	D.W shoot (g)/plant
Control	38.33^{e}	6.26^{d}	31.00^{e}	100.60^{e}	2.82^{d}	25.11^{g}	7.53^{d}
GA_3 100 ppm	53.83^{d}	8.63^{cd}	45.00^{d}	246.70^{cd}	4.48^{bc}	44.48^{e}	13.34^{c}
GA_3 200 ppm	60.08^{bc}	11.08^{abc}	57.67^{ab}	312.70^{bc}	4.67^{bc}	49.27^{d}	16.25^{b}
GA_3 300 ppm	71.33^{a}	12.55^{a}	59.00^{a}	373.80^{ab}	5.01^{b}	75.41^{a}	21.86^{a}
ME 200 ppm	58.2^{cd}	9.437^{bc}	53.67^{bc}	300.60^{cd}	4.82^{bc}	57.11^{c}	16.56^{b}
ME 400 ppm	55.17^{cd}	9.55^{bc}	49.00^{cd}	232.50^{d}	4.33^{c}	40.16^{f}	12.04^{c}
ME 600 ppm	69.37^{ab}	11.36^{ab}	56.00^{ab}	396.90^{a}	5.64^{a}	67.07^{b}	20.12^{a}

According to Duncan's 1955 test with correction ($P \leq 0.05$), means in each column with the same letter (s) are not significantly different. Comparing individual treatment using upper case letters (a-g). GA_3* Gibberellic acid; ME* Methionine.

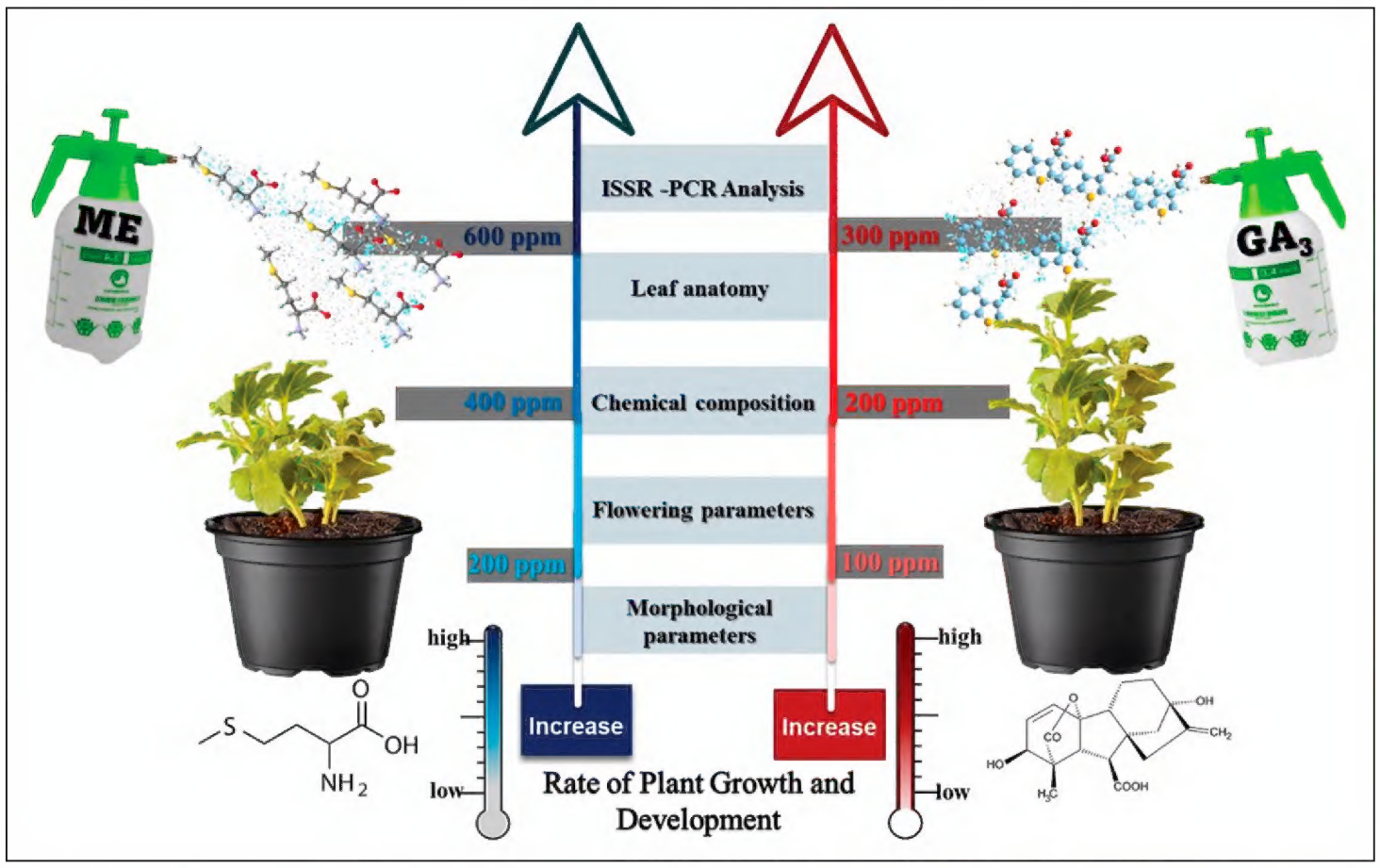

Fig 1. Effect of different concentrations of gibberellic acid (GA_3) and methionine (ME) on different parameters of *Chrysanthemum morifolium* Ramat "Zambla yellow" plant during the experiment.

gibberellic acid increases cell elongation, cell division, and cell wall plasticity, that in turn releases intermodal length. However, Sumi et al. (2021) reported that treating *Brassica juncea* L. with 50 ppm GA_3 increased most of the vegetative parameters. In addition, Aboud and Abd-Alrahman, (2020)reported that foliar application of ME effect phytohormones, which could increase the photosynthetic activity, leading to better growth physical quality (length, diameter, fresh, dry weights) and yield. In another study, the influence of ME concentrations induced a significant stimulation on the biomass accumulation and growth as a result of the positive effects of ME on the biosynthesis and concentration and also the root growth and elongation of the plant (Mahmoud and Abdelhameed, 2021). The positive influence of GA_3 on enhancing cell division and elongation of new cells formed in the plants, as well as slight variations in the diameter of stem for differences in GA_3levels, could be enhanced intermodal lengths and cell elongation in treated plants (Miceliet al., 2019). In another study, Phytohormones, particularly GA_3, are important growth hormones that regulate a variety of physiological processes, such as plant growth and composition, leaf expansion stimulation, flowering, elongation, and osmoregulation stimulation in internodes, germination, dry matter, and biomass composition (Karki et al., 2021).

Several amino acids, such as methionine, positively impact plant growth, yield, and flowering, through their effects on metabolism, enzymatic and non-enzymatic activities, and hormonal interactions. ME is a group of essential amino acids that play several roles in different metabolic processes. ME a significant role in the biosynthesis of cytokinin, auxins, and brassinosteroids (El-Awadi et al., 2011 and Yong et al., 2014).

Flowering parameters

Data in (Table 4) showed that both gibberellic acid and methionine significantly influencedthe flowering parameters of *Chrysanthemum morifolium* cv "Zambla yellow" plants. The number of flowers, stalk length (cm), fresh flower weight and flower dry weight were significantly increased by the successive increase in the concentration of gibberellic acid. The result indicates that the highest concentration of GA_3 (300 ppm) was more effective on the number of flowers/plant (24.56 and 22.30) and stalk length (cm)/plant (90.7 cm, 85.63 cm). In addition, the highest application of methionine (600 ppm) gave the highest value of the diameter of flowers (cm) (8.62 and 8.04 cm), time to showing color days (138.83 and 135.33 days), fresh flower weight (9.84 and 8.13 g), and flower dry weight (1.48 and 1.22 g) in both seasons, respectively). In contrast, the lowest values of most flower parameters were obtained from the control plants. Moreover, various levels of $GA_3$and ME application significantly showed variation for days to flower initiation of chrysanthemum plants. The minimum days were obtained from plants spraying with the highest concentration of GA_3 (300 ppm). These results are consistent with those found in (Zarrinet al., 2021) they reported that treatments of 300 mg/l GA_3 produced the greatest number of flower and dry weight of flowers. However, treated chrysanthemum plants with 300 mg/l GA_3 decreased the days to flower compared with control and other concentration of GA_3. Also, Acharyaet al., (2021) found that using GA_3 at 300 ppm on African

Table 4: Impact of different concentrations of gibberellic acid and methionine on the flowering parameters of *Chrysanthemum morifolium*, Ramat "Zambla yellow" plant during 2021 and 2022 seasons

Treatments	First season					
	No.of flowers/ plant	diameter of flowers (cm)	Time to showing color (days)	Flower stalk length (cm)/plant	F.W. flowers (g)	D.W. Flowers (g)
Control	11.17^{e}	4.89^{e}	133.51^{b}	51.87^{e}	4.20^{d}	0.63^{c}
GA_3 100 ppm	18.44^{c}	6.88^{d}	118.40^{d}	67.58^{d}	6.13^{c}	0.919^{bc}
GA_3 200 ppm	21.98^{b}	7.13^{cd}	116.33^{de}	74.23^{c}	7.93^{b}	1.315^{a}
GA_3 300 ppm	24.56^{a}	8.03^{ab}	114.33^{e}	90.7^{a}	8.77^{ab}	1.189^{ab}
ME 200 ppm	17.74^{c}	7.61^{bc}	129.33^{c}	70.81^{d}	6.09^{c}	0.913^{bc}
ME 400 ppm	15.09^{d}	6.74^{d}	131.17b^{c}	60.92^{e}	5.87^{cd}	0.880^{bc}
ME 600 ppm	22.63^{ab}	8.62^{a}	138.83^{a}	85.09^{b}	9.84^{a}	1.476^{a}
	Second season					
	No. of flowers/ plant	diameter of flowers (cm)	Time to showing color (days)	Flower stalk length (cm)/plant	F.W. flowers (g)	D.W. Flowers (g)
Control	10.25^{e}	4.48^{e}	132.00^{bc}	45.14^{e}	3.90^{f}	0.585^{f}
GA_3 100 ppm	16.38^{c}	6.45^{cd}	115.50^{d}	62.4^{d}	5.88^{d}	0.882^{d}
GA_3 200 ppm	20.32^{b}	6.88^{b-d}	114.00^{de}	70.61^{c}	6.80^{c}	1.02^{c}
GA_3 300 ppm	22.30^{a}	7.52^{ab}	112.17^{e}	85.63^{a}	7.36^{b}	1.104^{b}
ME 200 ppm	16.56^{c}	7.15^{a-c}	130.67^{c}	67.68^{d}	5.90^{d}	0.885^{d}
ME 400 ppm	13.55^{d}	5.95^{d}	133.97^{ab}	62.42^{e}	5.23^{e}	0.784^{e}
ME 600 ppm	20.88^{ab}	8.04^{a}	135.33^{a}	81.04^{b}	8.13^{a}	1.219^{a}

According to Duncan's 1955test with correction ($P \leq 0.05$), means in each column with the same letter (s) are not significantly different. Comparing individual treatment using upper case letters (a-g). GA_3* Gibberellic acid; ME* Methionine

marigold *(Tageteserecta)* showed that increasing the number of flowers and earlier days to 50% flowering. Furthermore, treated plants with 250,350 ppm gave the greatest fresh weight of flowers (Jokar and Hassanpour Asil, 2021) demonstrated that foliar application with 300 ppm GA_3 has enhanced growth and accelerated flowering. Mzabri et al., (2021) reported that treated corms of saffron with 100 and 200 ppm GA_3before planting resulted in the greatest number of flower and increased yield. The same results were found in other studies, on carnation (Kumar et al., 2003), Lilium (Mojtahedi et al., 2014), and gladioli (Padmalathaet al.,2013). These correlations among flowering parameters include the number of flowers and diameter of flowers and morphological parameters such as total leaf area, plant height underline the chlorophyll content play in the productivity and precocity of chrysanthemum (Mollafilabi, 2004; Douglas et al., 2014 and Ameri et al., 2019).

The observation effect might be attributed to the influence of GA_3on the reduction of the abscisic acid level in buds, which floral induction initiation and thus accelerates flowering (Phengphachanhet al., 2012).Therefore, the important role of GA_3in this physiological activity is increasing starch catabolism resulting in simple sugars (Chrungoo and Farooq, 1991).

Several amino acids, such as methionine, positively impact on plant growth, yield, and flowering, through their effects on metabolism, enzymatic and non-enzymatic activities (Yong, et al., 2014).

Chemical composition

Figs. 1, 2, and 3 showed the effect of gibberellic acid and methionine on the chemical composition of C. *morifolium* Ramat "Zambla yellow" plants. Our research found that chlorophyll a and b, total chlorophylls, carotenoids, K%, P%, total carbohydrates content, and total phenols were recorded at the highest values from the treatment of GA_3 at the rate of 300 ppm. Alternatively, the lowest values of chlorophyll a and b, total chlorophylls, carotenoids, K%, P%, carbohydrate content, and total phenols were obtained from the control plants. Moreover, plants treated with ME at 600 ppm gave the highest values of N% and protein compared to the other treatments and control. The findings of this study were consistent with those of (Graham and Ballesteros, 1980) they found that applying GA_3caused the plants to accumulate ascorbic acid β- carotene, soluble carbohydrates, proteins, and starch. Atteyaet al. (2018)stated the treated jojoba plant with 150 ppm GA_3gave the greatest percentage of total chlorophylls, and mineral % content. Foliar application with 200 ppm GA_3 led to a positive increase in the total chlorophyll content compared with untreated chrysanthemum plants (Gabrel et al.,2018). In addition, using ME increased chlorophyll and carotenoids content in plant leaves (Hashimoto et al., 2015).

These findings could be attributed to the application of GA_3, causing active enzymes needed for chlorophyll accumulation and decreasing chlorophyll degradation (Gabrel et al.,2018). The treatment with ME induced a positive effect and improvement on the biomass,

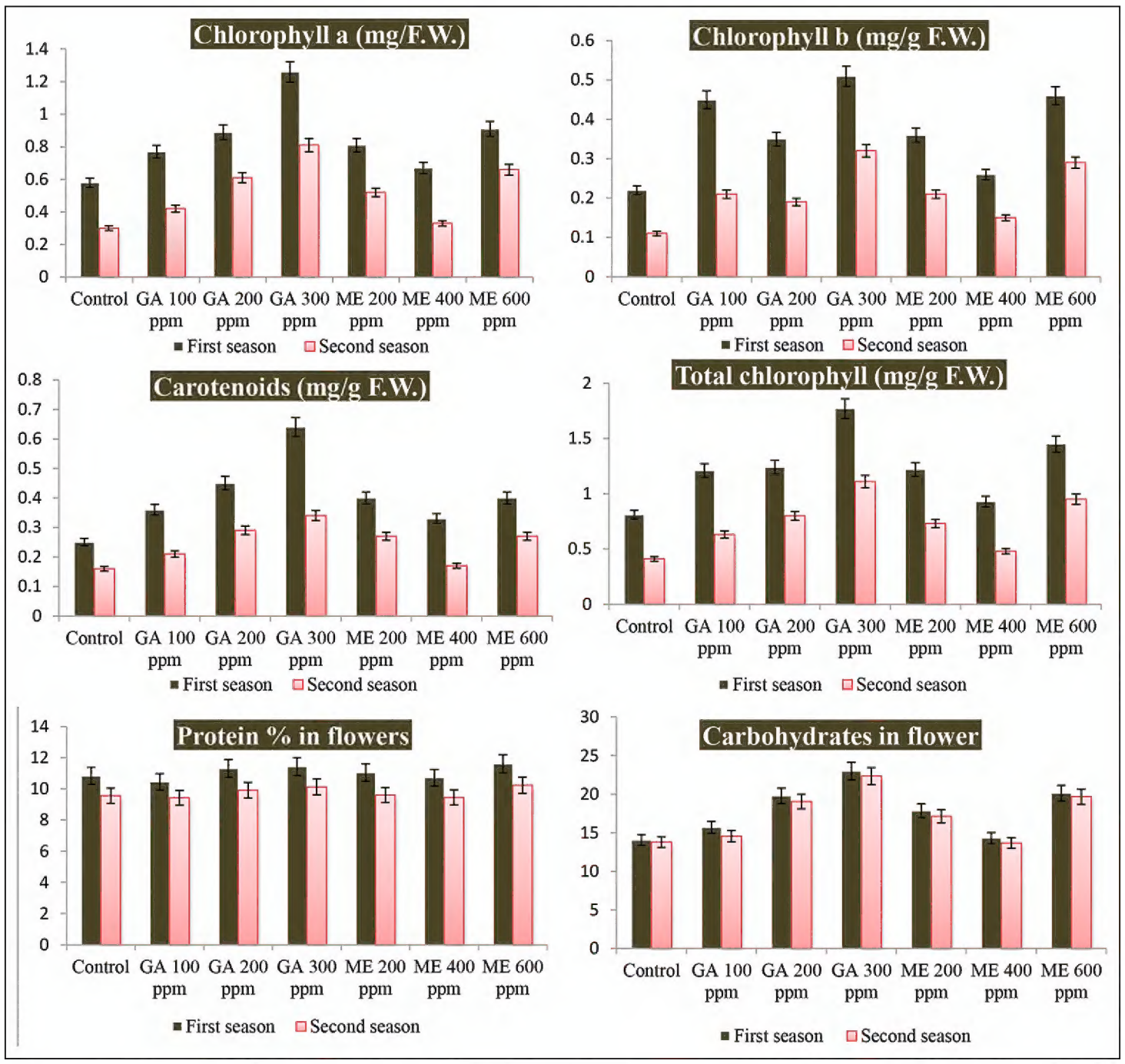

Fig 2. Impact of different concentrations of gibberellic acid and methionine on chlorophyll content in leaves and some chemical composition in flowers of *Chrysanthemum morifolium* Ramt "Zambla yellow" plant during 2021 and 2022 seasons.

growth, photosynthetic pigment content, total protein, and antioxidant activities in plants, which could be due to their significant effects on synthesis concentration and elongation of the plant (Mahmoud and Abdelhameed, 2021).

Leaf anatomy

- Leaf anatomical structure under the normal conditions

C. *morifolium* leaf is a dorsiventral type; this means that the palisade tissue is located on the adaxial (upper) side of the blade and the spongy tissue on the abaxial (lower) one. Trichomes are observed on both surfaces. The palisade tissue consists of 1-2 layers of cells; these cells are vertically arranged, parallel to each other and have many chloroplasts. The spongy tissue comprises several layers of parenchymatous loosely arranged cells with intercellular spaces. The vascular bundle of the main vein is large and consists of xylem lying towards the upper epidermis and phloem towards the lower epidermis.

- Effect of GA_3 and ME on leaf anatomical measurements

Table 5 and Fig. 4 showed the effect of spraying with GA_3, and ME as growth Stimulants at different concentrations on the anatomical measurements of *C. morifolium* Ramat "Zambla Yellow" leaves.

The data in Table 5 indicate that the foliar application of GA_3 and ME improved all studied traits except the treatment with ME at a concentration of 400 ppm approximately equal or less than the control in some leaf anatomical measurements. Regarding the treatment with GA_3, the measurements of the leaf anatomy increased by increasing the concentration of GA_3 under study, as the concentration of 300 ppm achieved the highest value for both the thickness of the main vein and lamina of the leaf blade, which was 1235.22 and 812.01 µm; respectively compared to the control (886.81, 567.87µm). In this concern, Eriksson et al.(2006) mentioned that GA_3 promotes cell elongation and can regulate shoot elongation in Arabidopsis. Moreover, GA_3 participates in leaf expansion and plant growth (Serraniet al., 2007).

In the case of ME treatment, there was an improvement in most characteristics, especially at a concentration of

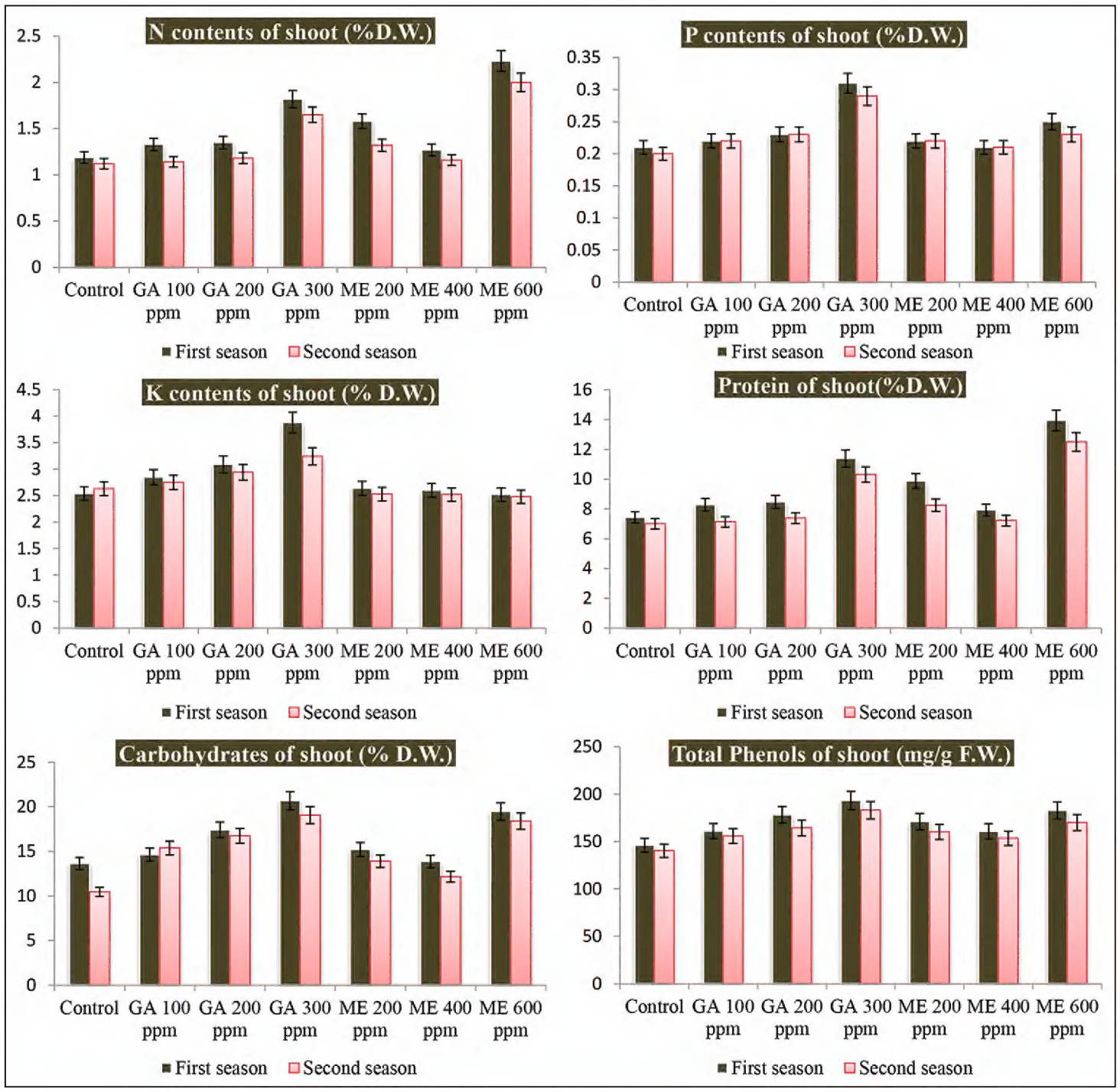

Fig 3. Impact of different concentrations of gibberellic acid and methionine on some chemical composition in shoots of *Chrysanthemum morifolium* Ramat "Zambla yellow" plant during 2021 and 2022 seasons.

Table 5: Effect of different concentrations of gibberellic acid and methionine on leaf anatomy measurements (µm) of *Chrysanthemum morifolium* Ramat "Zambla yellow

Characters (µm)	Treatments						
	Control	Gibberellic acid (ppm)			Methionine (ppm)		
		100	200	300	200	400	600
Upper epidermis	33.75	37.66	38.93	39.75	30.18	27.24	30.94
Main vein thickness	886.81	1045.13	1097.89	1235.22	1105.04	884.05	1155.35
Lamina thickness	567.87	609.31	645.21	812.01	538.82	529.23	575.15
Palisad tissue thickness	181.16	195.30	218.70	241.21	196.48	163.27	203.65
Spongy tissue thickness	375.71	397.13	422.19	566.97	333.80	299.18	366.34
Dimensions of vascular bundle • Length	288.28	349.61	405.03	467.05	390.11	331.26	465.86
• Width	293.70	320.72	329.28	483.84	412.51	347.32	576.36
Xylem tissue thickness	145.07	175.81	146.47	282.36	194.55	160.03	208.06
Phloem tissue thickness	47.05	79.44	81.58	88.36	64.02	64.08	81.88
Lower epidermis	24.00	26.37	29.24	32.52	29.50	24.62	25.15

600 ppm, which recorded the highest measurements of the leaf anatomy; like main vein and lamina thickness, dimensions of vascular bundle and thickness of both xylem, and phloem tissues compared with control and other concentrations. Moreover, the vascular bundle in the midrib region increased in size due to the increase in the length and width of the bundle in all concentrations of $GA_3$and ME. Also, the thickness of xylem tissue increased in all tested concentrations, responsible for transporting the water and solute. It achieved 175.81, 146.47, and 282.36 µm for GA_3concentrations, respectively, as well as 194.55, 160.03, and 208.06 µm for ME concentrations,

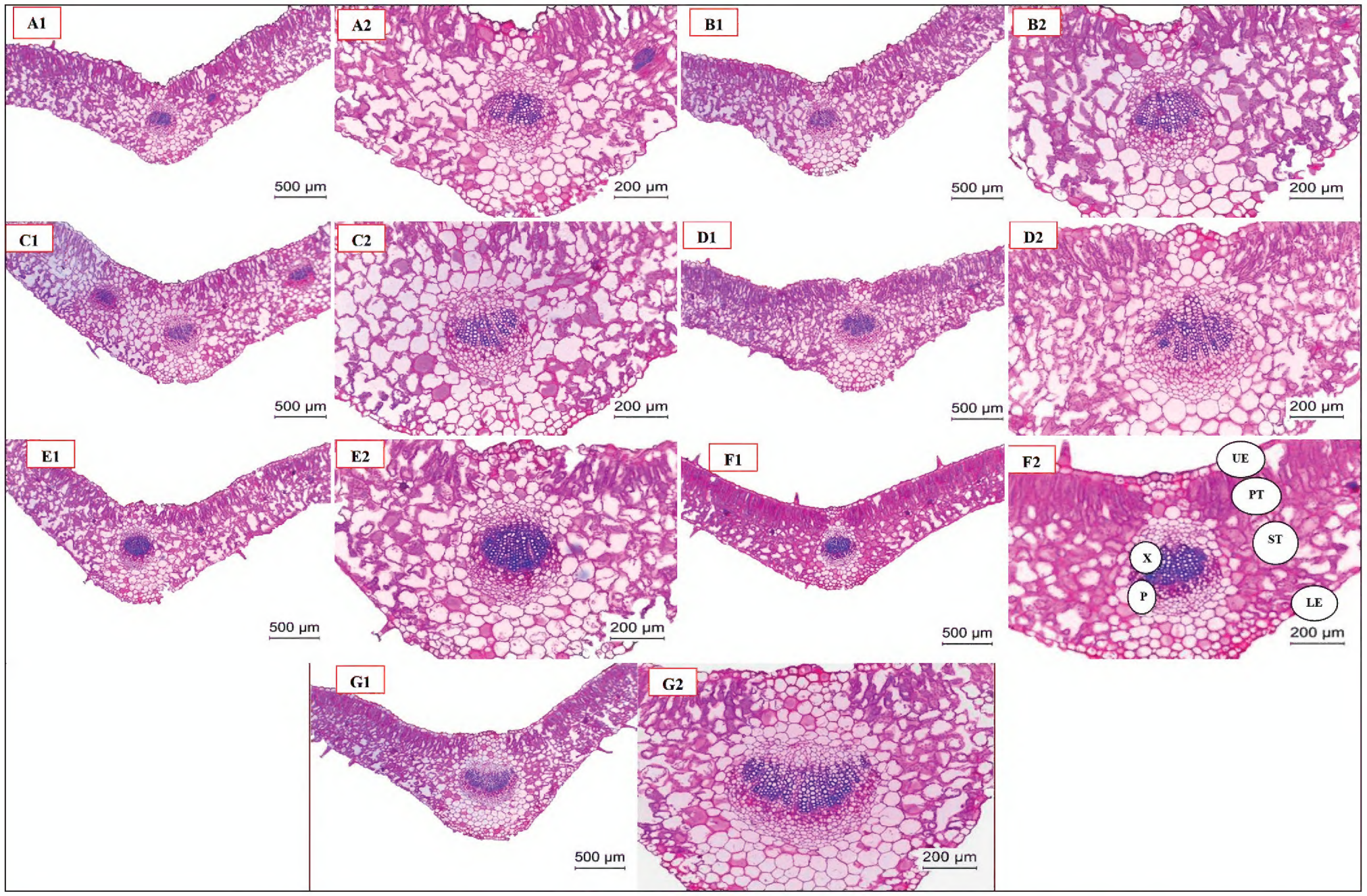

Fig 4. Cross sections of *Chrysanthemums morifolium* Leaves. (A) Untreated plant; (B, C, D) leaves treated with gibberellic acid at 100, 200 and 300 ppm; (E, F, G) leaves treated with methionine at 200, 400 and 600 ppm. From A1 to G1; whole sections of leaves at scale bars 500 µm. from A2 to G2; magnification portions through midrib region at scale bars 200 µm.
LE; lower epidermis, P; phloem, PT; palisad tissue, ST; spongy tissue, UE; upper epidermis, X; xylem.

respectively, compared to control, which scored 145.07µm. Ragniet al., (2011) indicated that mobile GA_3 promotes xylem expansion in the hypocotyl of Arabidopsis. Akram et al., (2020)reported that exogenous application of L- methionine improved the anatomical features of bitter gourd plant under drought conditions, as it increased leaf vascular bundle area and increased leaf xylem and phloem area. It is clear from the previous data that the results of morphological traits were in harmony with the results of anatomical studies, and that was verified by increasing most characters under study through treatment with $GA_3$and ME that logic from results obtained by morphology and histology.

ISSR -PCR analysis

Ten ISSR primers were designed to distinguish between the two chrysanthemum treatments. These primers produced an accurate result with distinct polymorphisms and clear resolution within the chrysanthemum. These ISSRs primers have generated a total of 128 bands that were detected a ranging from10 (for ISSR9 and 11) to 16 (for ISSR6), Table 6. Polymorphism percentages ranged from (8 to 43%). Polymorphic bands can benefit from treatment differentiation in two ways: the first is through the release of distinct banding patterns to individual species, and the second is through the presence or absence of distinctive band (s) (marker bands) that distinguish an individual from its population, so establishing its DNA fingerprint is critical. Primer ISSR-4 and ISSR-11 amplify the maximum number of unique bands, recording four and three, respectively, while ISSR-2 and ISSR-9 no unique bands were amplified. ISSR primers were robust and informative, indicating that they might be a better tool for genetic diversity and phylogenetic analysis, as shown in Table 6, and Fig. 5.The treatment with GA_3 increased the expression of genes linked to DNA replication (histones h1 and h2b) and cell elongation (expansion and -tubulin), which could explain the differences in ISSR banding patterns. We propose that using GA_3 and ME induces complicated epigenetic re-programming, which could result in different developmental phenotypes. These findings could be helpful for future research into epigenetic mechanisms in other important ornamental plants. As shown in Table 7 and Fig. 6 a dendrogram illustrated two distinct groups. The first group

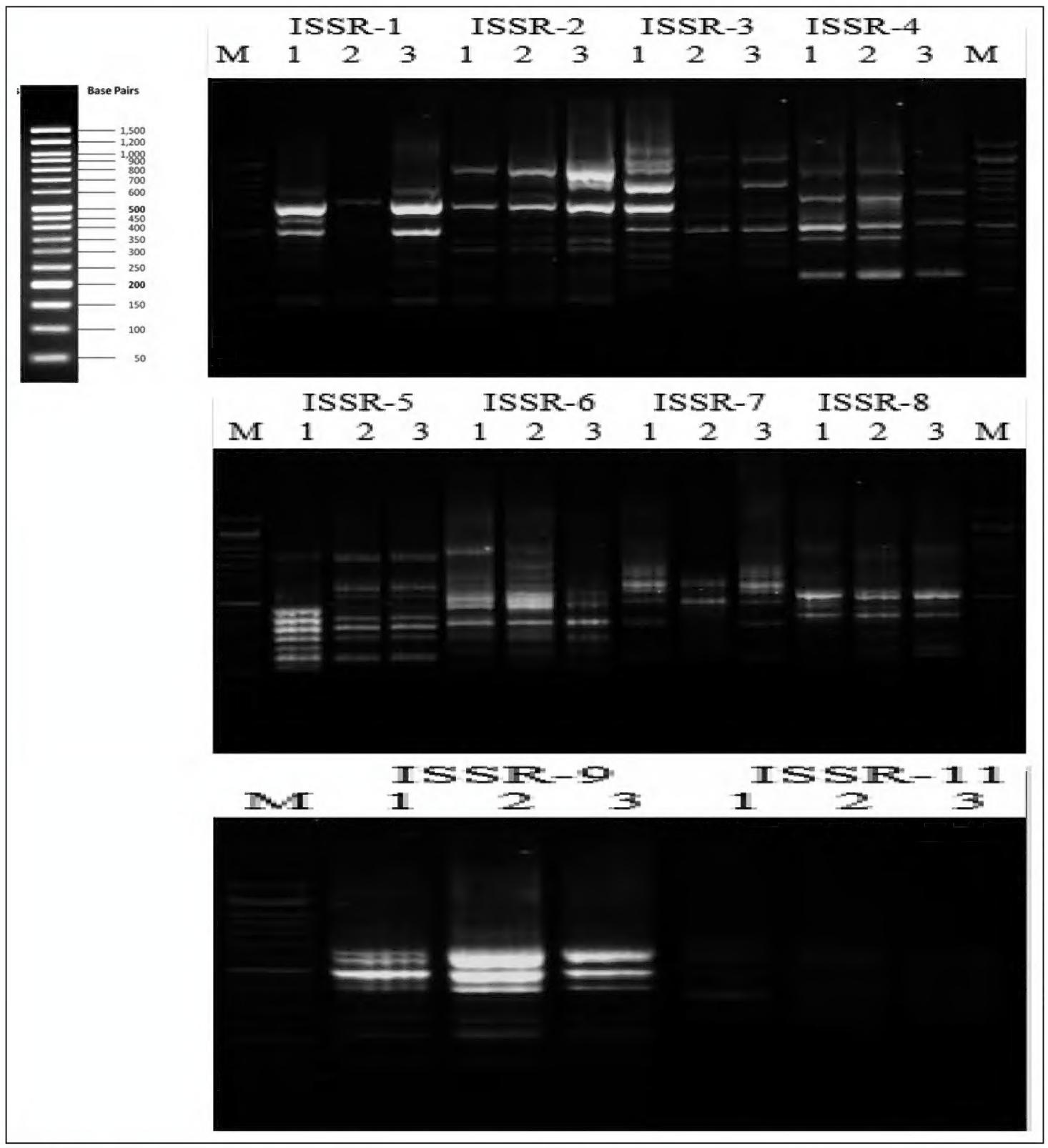

Fig 5. PCR amplification using issr primer combinations of control, GA_3 at 300ppm, and ME 600ppm (1–3), M (DNA ladder 100 bp) of *Chrysanthemums morifolium* plant.

Table 6: The statistical analysis of ISSR primers used to discriminate between

No	Name of primer	Monomorphic bands	Polymorphic bands	Number of unique bands	Total bands	Polymorphism (%)	MW range (bp)	Mean of frequency
1	ISSR-1	8	4	2	14	43	200-1500	0.8
2	ISSR-2	12	1	0	13	8	200-1100	0.9
3	ISSR-3	10	3	1	14	29	200-1500	0.8
4	ISSR-4	7	1	4	12	42	200-1500	0.8
5	ISSR-5	9	2	1	12	25	200-900	0.9
6	ISSR-6	12	2	2	16	25	230-1000	0.9
7	ISSR-7	10	1	2	13	23	250-1000	0.9
8	ISSR-8	11	2	1	14	21	200-1000	0.9
9	ISSR-9	9	1	0	10	10	150-1100	0.9
10	ISSR-10	6	1	3	10	40	250-800	0.8
Total		94	18	16	128			8.6
Average		9.4	1.8	1.6	12.8			0.86

included one cluster with control *C.morifolium* cv "Zambla Yellow" (No.1), with a genetic similarity ratio of 88% with (GA_3 300 ppm, and ME 600 ppm). On the other hand, the second group included GA_3 300 ppm and ME 600 ppm (No.2 and 3) respectively with different distances between them. After exposure, *Tulip agesneriana* L. to various dosages of gamma rays(Li et al., 2022)performed the ISSR-PCR genetic marker to detect mutants of phenotypic variation

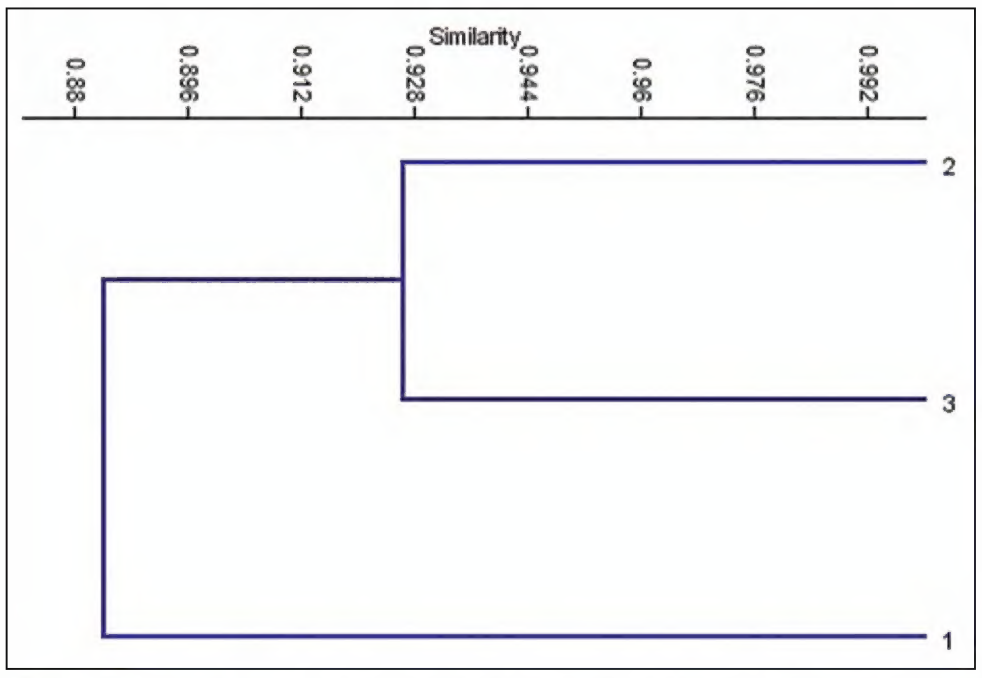

Fig 6. Dendrogram showing the relationships between *Chrysanthemum morifolium* Ramat"Zambla yellow" (control) and the potent treatments generated from ISSR data following the analysis of control and two treatments of *Chrysanthemum morifolium*cv "Zambla yellow**1**: Control (without any treatment); **2**: plants treated with GA 300ppm; **3**; plants treated with ME 600 ppm.

Table 7: The similarity matrix between control and GA_3 300 ppm as well as ME 600 ppm treatments of *the* chrysanthemum plant

	Similarity matrix between		
Name	**Control**	**GA 300ppm**	**ME 600 ppm**
Control	100		
GA 300 ppm	88	100	
ME 600 ppm	88	93	100

GA: gibberellic acid ME: Methionine

plants. Polymorphic genetic markers, which can distinguish varieties and parents, are commonly utilized in plant development efforts. ISSR has been frequently used to discover DNA polymorphisms in *Pimpinella anisum* L. (Akçali Giachino, 2020) and on *Populus alba* (Salimet al., 2019), and *Solidago canadensis* cv, Tara plants (El-Sayed et al., 2020).

In comparison with SCoT markers, ISSR markers were more efficient in assessing genetic diversity and produced more polymorphic bands, according to certain studies (Xanthopoulou et al., 2015 and Kobeissiet al., 2019). Another study confirmed the efficiency of several ISSR, markers for the analysis of genetic diversity and establishing genetic relationships among control and their treatments, it is well known that multilocus DNA markers (such as ISSRs) are often transferable to different plant species and genera; however, the efficiency of their application can vary significantly depending on plant species (Samarinaet al., 2021; El-Sayed et al.,2021).

CONCLUSIONS

Based on the results of this work, it seems that higher concentrations of gibberellic acid and methionine had a positive impact on growth such as plant height, stem diameter, number of leaves, and fresh and dry weight. Moreover, flowering parameters, chemical composition, leaf anatomical structure, and detection of genetic diversity of *Chrysanthemum morifolium* Ramat "Zambla yellow". Therefore, the treatment of 300ppm GA_3 and ME 600ppm can be a potential treatment for commercial production of chrysanthemum flowers.

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Author contributions
E.Z.O., R.A.E.Z. and I.M.E.S. conceived the experiments and methodology; E.Z.O., R.A.E.Z., H.M.F. and I.M.E.S performed the experiments, measurements, and analyses required; E.Z.O., I.M.E.S. and H.M.F. curated and analyzed the data; I. M.E.S., and R.A.E.Z. wrote the manuscript draft; I.M.E.S., and R.A.E.Z. reviewed, edited, and completed the manuscript. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Aboud, F. S. and H. A. Abd-Alrahman. 2020. Effect of foliar spray with some growth stimulants on potato plants under different growing media. Plant Arch. 20: 9244-9252.

Acharya, S., B. Ghimire, S. Gaihre, K. Aryal and L. B. Chhetri. 2021. Effect of gibberellic acid on growth and flowering attributes of African marigold (*Tageteserecta*) in inner terai of Nepal. J. Agric. Nat. Resour. 4: 134-147.

Akçali Giachino, R. R. 2020. Investigation of the genetic variation of anise (*Pimpinellaanisum* L.) using RAPD and ISSR markers. Genet. Resour. Crop Evol. 67: 763-780.

Akram, N. A., E. H. Umm, M. Ashraf, M. Ashraf and M. Sadiq. 2020. Exogenous application of L-methionine mitigates the drought-induced oddities in biochemical and anatomical responses of bitter gourd (*Momordica charantia* L.). Sci. Hortic. 267: 109333.

Albadwawi, M. A. O. K., Z. F. R. Ahmed, S. S. Kurup, M. A. Alyafei, A. A. Jaleel. 2022. Comparative evaluation of aquaponic and soil

systems on yield and antioxidant levels in Basil, an important food plant in *Lamiaceae*. Agronomy. 12: 3007.

Alziyituni, H. O. M. 2023. Effect of humic Acid and gibberellic acid on growth, flowering and chemical composition of African marigold (*Tageteserecta*). J. Adv. Agric. Res. 28(1): 193-201.

Ameri, R., M. Aziziand and A. Mollafilabi. 2019. Effects of gibberellic acid and naphthalene acetic acid on saffron plant (*Crocus Sativus* L.) under field conditions. J. Appl. Hortic. 21: 123-130.

Amir R., G. Galili, and H. Cohen. 2018. The metabolic roles of free amino acids during seed development. Plant Sci. 275: 11-18.

Amir, R., H. Cohen and Y. Hacham. 2019. Revisiting the attempts to fortify methionine content in plant seeds. J. Exp. Bot. 70: 4105-4114.

Amir, R., T. Han and F. Ma. 2012. Bioengineering approaches to improve the nutritional values of seeds by increasing their methionine content. Mol. Breed. 29: 915-924.

Atteya, A. K. G., S. K. Al-Taweel, E. A. E. Genaidy and H. A. Zahran. 2018. Effect of gibberellic acid and zinc sulphate on vegetative, flowering, seed yield and chemical consistent of Jojoba plant (*Simmondsia chinensis*). Indian J. Agric. Res. 52: 542-547.

Chrungoo, N. K. and S. Farooq. 1991. Effect of GA3 and NAA on certain nitrogen fractions in corms of saffron crocus (*Crocus-Sativus* L.) during development. Acta. Physiol. Plant. 13: 159-165.

Douglas, M. H., B. M. Smallfield, A. R. Wallace and J. A. Mcgimpsey. 2014. Saffron (*Crocus sativus* L.): The effect of mother corm size on progeny multiplication, flower and stigma production. Sci. Hortic. 166: 50-58.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350-356.

Duncan, B. D. 1955. Multiple range and multiple F tests. Biometrics. 11: 1-42.

El-Awadi, M., A. El-Bassiony, Z. Fawzy and M. El-Nemr. 2011. Response of snap bean (*Phaseolus vulgaris* L) plants to nitrogen fertilizer and foliar application with methionine and tryptophan. Nat. Sci. 9: 87-94.

EL-Bauome, H. A., E. A. Abdeldaym, M. A. Abd El-Hady, D. B. E. Darwish, M. S. Alsubeie, M. M. El-Mogy and S. M. Doklega. 2022. Exogenous proline, methionine, and melatonin stimulate growth, quality, and drought tolerance in cauliflower plants. Agriculture, 12(9): 1301.

El-Sayed I. M., W.H. Salama, R. G. Salim and L. S. Taha. 2021. Relevance of nanoparticles on micropropagtion, antioxidant activity and molecular characterization of *Sequoia sempervirens* L. Plant. Jordan J. Biol. Sci. 14: 373-82.

El-Sayed, I. M. and R. A. El-Ziat. 2021. Utilization of environmentally friendly essential oils on enhancing the postharvest characteristics of *Chrysanthemum morifolium* ramat cut flowers. Heliyon. 7: e05909.

El-Sayed, I. M., R. G. Salim, E. F. El-Haggar, R. A. El-Ziat and D. M. Soliman. 2020. Molecular characterization and positive impact of brassinosteroids and chitosan on *Solidago canadensis* cv. Tara characteristics. Horticulturae. 6: 100.

Eriksson, S., H. Böhlenius, T. Moritz and O. Nilsson. 2006. GA_4 is the active gibberellin in the regulation of leafy transcription and arabidopsis floral initiation. Plant Cell. 18: 2172-2181.

Gabrel, F., K. Mahmoud and E. N. Ali. 2018. Effect of benzyl adenine and gibberellic acid on the vegetative growth and flowering of chrysanthemum plant. Alex. J. Agric. Sci. 63: 29-40.

Galili, G., R. Amir and A. R. Fernie. 2016. The regulation of essential amino acid synthesis and accumulation in plants. Annu. Rev. Plant Biol. 67: 153-178.

Graham, H. D. and M. Ballesteros. 1980. Effect of plant growth regulators on plant nutrients. J. Food Sci. 45: 502-505.

Hammer, O., D. A. T. Harper and P. D. Ryan. 2001. Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4: 9-18.

Hashimoto, H., M. Sugisaki and M. Yoshizawa. 2015. Ultrafast time-resolved vibrational spectroscopies of carotenoids in photosynthesis. Biochim. Biophys. Acta. 1847: 69-78.

Hassan, F. E., M. A. S. Alyafei, S. Kurup, A. Jaleel, N. Al Busaidi, Z. F. R. Ahmed. 2023. Effective priming techniques to enhance ghaf (*Prosopis cineraria* L. Druce) seed germination for mass planting. Horticulturae. 9: 542.

Ibrahim, E.A. 2021. Genetic diversity in Egyptian bottle gourd genotypes based on ISSR markers. Ecol. Genet. Genom. 18: 100079.

Jokar, N. K. and M. Hassanpour Asil. 2021. Effect of gibberellic acid and vermicompost on growth and flowering of daffodil flower. J. Crop Improv. 23: 183-198.

Karki, P., P. N. Atreya and S. Shrestha. 2021. Effect of maleic hydrazide and gibberellic acid on growth and yield of African marigold (*Tageteserecta* L.) cv. Calcuttia orange. Fundam. Appl. Agric. 6: 272-278.

Khalil, H.A., D. O. El-Ansary and Z. F. R. Ahmed. 2022. Mitigation of salinity stress on pomegranate (*Punicagranatum* L. cv. wonderful) plant using salicylic acid foliar spray. Horticulturae. 8: 375.

Khan, N., S. Ali, P. Zandi, A. Mehmood, S. Ullah, M. Ikram, M. A. S. Ismail and M. Babar. 2020. Role of sugars, amino acids and organic acids in improving plant abiotic stress tolerance. Pak. J. Bot. 52: 355-363.

Kirmizi, S., G. Guleryuz and H. Arslan. 2017. Effects of environmental and storage conditions on the germination of allium species. Fresenius Environ. Bull. 26: 3470-3478.

Kobeissi, B., A. Saidi, A. Kobeissi and M. Shafie. 2019. Applicability of SCoT and SSR molecular markers for genetic diversity analysis in *Chrysanthemum morifolium* genotypes. Proc. Natl. Acad. Sci. India Sect. B Boil. Sci. 89: 1067-1077.

Krishnan, H. B. and J. M. Jez. 2018. Review: The promise and limits for enhancing sulfur-containing amino acid content of soybean seed. Plant Sci. 272: 14-21.

Kumar, C., R. Sharma and A. K. Bachhawat. 2003. Investigations into the polymorphisms at the ECM38 locus of two widely used saccharomyces cerevisiae S288C strains, YPH499 and BY4742. Yeast. 20: 857-863.

Li, Y., L. Chen, X. Zhan, L. Liu, F. Feng, Z. Guo, D. Wang and H. Chen. 2022. Biological effects of gamma-ray radiation on tulip (*Tulip agesneriana* L.). Peer J. 10: e12792.

Liu, H., C. Luo, D. Chen, Y. Wang, S. Guo, X. Chen, J. Bai, M. Li, X. Huang, X. Cheng and C. Huang. 2021. Whole-transcriptome analysis of differentially expressed genes in the mutant and normal capitula of *Chrysanthemum morifolium*. BMC Genomic Data. 22: 2.

Maeda, H. and N. Dudareva. 2012. The shikimate pathway and aromatic amino acid biosynthesis in plants. Annu. Rev. Plant Biol. 63: 73-105.

Mahmoud, N. E. and R. M. Abdelhameed. 2021. Superiority of modified graphene oxide for enhancing the growth, yield, and antioxidant potential of pearl millet (*Pennisetum glaucum* L.) under salt stress. Plant Stress. 2: 100025.

Mehak, G., N. A. Akram, M. Ashraf, P. Kaushik, M. A. El-Sheikh and P. Ahmad. 2021. Methionine-induced regulation of growth, secondary metabolites and oxidative defense system in

sunflower (*Helianthus annuus* L.) plants subjected to water deficit stress. Plos One. 16(12): e0259585.

Miceli, A., A. Moncada, L. Sabatino and F. Vetrano. 2019. Effect of gibberellic acid on growth, yield, and quality of leaf lettuce and rocket grown in a floating system. Agronomy. 9: 382.

Mojtahedi, N., P. Koobaz, M. Fathi, O. Dabirashrafi, P. Azadi and S. Khosravi. 2014. Maturating, enlarging and breaking dormancy of *In vitro Lilium* Bulblets. Int. J. Hortic. Sci. Technol. 1: 101-109.

Mollafilabi, A. 2004. Experimental findings of production and echo physiological aspects of saffron (*Crocus Sativus* L.). Acta Hortic. 650: 195-200.

Mzabri, I., M. Rimani, N. Kouddane and A. Berrichi. 2021. Study of the effect of pretreatment of corms by different concentrations of gibberellic acid and at different periods on the growth, flowering, and quality of saffron in Eastern Morocco. Adv. Agric. 2021: 9979827.

Nassar, M. A. and K. F. El-Sahhar. 1998. Botanical Preparations and Microscopy (Microtechnique). Academic Bookshop: Giza, Egypt.

Padmalatha, T., G. S. Reddy, R. Chandrasekhar, A. S. Shankar and A. Chaturvedi. 2013. Effect of pre-planting soaking of corms with chemicals and plant growth regulators on dormancy breaking and corm and cormel production in gladiolus. Int. J. Plant Anim. Env. Sci. 3: 28-33.

Pang, X. M., Z. Y. Zhang, X. P. Wen, Y. Ban and T. Moriguchi. 2007. Polyamines, all-purpose players in response to environment stresses in plants. Plant Stress. 1: 173-188.

Phengphachanh, B., D. Naphrom, W. Bundithya and N. Potapohn. 2012. Effects of day-length and gibberellic acid (GA3) on flowering and endogenous hormone levels in *Rhynchostylisgigantea* (Lindl.) Ridl. J. Agric. Sci. 4: 217-222.

Ragni, L., K. Nieminen, D. Pacheco-Villalobos, R. Sibout, C. Schwechheimer and C. S. Hardtke. 2011. Mobile gibberellin directly stimulates Arabidopsis hypocotyl xylem expansion. Plant Cell. 23: 1322-1336.

Ravat, A. K. and M. Nirav. 2015. Influence of plant growth regulators on growth, seed yield and seed quality in okra [*Abelmoschus esculentus* (L.) Moench]. GAO-5 under middle Gujarat condition. Int. J. Agric. Sci. 11: 51-157.

Rezaeian, Z., M. Haghighi and N. Kappel. 2022. The effect of spermidine and methionine application thorough two biosynthetic paths on flowering of early and late flowering genotypes of eggplant (*Solanummelongena* L.). Sci. Hortic. 306: 111459.

Roje, S. 2006. S-Adenosyl-l-methionine: Beyond the universal methyl group donor. Phytochemistry. 67: 1686-1698.

Salim, R. G., I. M. El-Sayed and L. S. Taha. 2019. Molecular characterization and *in Vitro* propagation Behavior of *Populus Alba* L. Plants under effect of slow growth conservation. Plant Arch. 19: 2443-2453.

Samarina, L. S., V. I. Malyarovskaya, S. Reim, L. G. Yakushina, N. G. Koninskaya, K. V. Klemeshova, R. M. Shkhalakhova, A. O. Matskiv, E. S. Shurkina, T. Y. Gabueva, N. A. Slepchenko and A. V. Ryndin. 2021. Transferability of ISSR, SCoT and SSR Markers for *Chrysanthemum* × *Morifolium* ramat and genetic relationships among commercial Russian cultivars. Plants (Basel). 10: 1302.

Saric, M. R., T. Kastrori-Cupina, I. Gergis. 1967. Chlorophyll Determination. Univ. Unoven Sadu Prakitikum is Kiziologize Bilika-Beagrad, Haucua Anjiga, p. 215.

Serrani, J. C., R. Sanjuán, O. Ruiz-Rivero, M. Fos and J. L. García Martínez. 2007. Gibberellin regulation of fruit set and growth in tomato. Plant Physiol. 145: 246-57.

Shah, S. H., and I. Ahmad. 2006. Effect of gibberellic acid spray on growth, nutrient uptake and yield attributes during various growth stages of black cumin (*Nigella sativa* L.). Asian J. Plant Sci. 5: 881-884.

Snell, F. D. and C. T. Snell. 1959. Colorimetric methods of analysis: Including photometric methods by forster dee snell. Van. Nostr. Soil Sci. 88: 59.

Soliman, D. M. and I. M. El-Sayed. 2023. Study postharvest characteristics, chemical composition and antimicrobial activity of *Dianthus caryophyllus* L., cut flowers using some essential oils. Ornament Hortic, 29: 37-47.

Soliman, D. M., A. M. A. Ahmed and I. M. El-Sayed. 2022. Lead toxicity and spermine as affecting the chemical composition and growth of *Solidago Canadensis* L. Cv. 'Tara' plant. Egypt. J. Chem. 65: 471-485.

Sumi, P., S. C. Meena, R. H. Meena and J. Choudhary. 2021. Effect of gibberellic acid and sulphur on growth and yield of mustard (*Brassica Juncea* L.). Pharma. Innov. J. 10: 1214-1217.

Swain, T. and W. E. Hillis. 1959. The phenolic constituents of *Prunus domestica*. I-the quantitative analysis of phenolic constituents. J. Sci. Food. Agric. 10: 63-68.

Taghipour, S., A. Ehtesham-Nia, H. Khodayari and H. Mumivand. 2021. Stability of yield and other important traits of chrysanthemum (*Chrysanthemum Morifolium* Ramat) cultivars using graphical Gge biplot method. J. Plant Prod. 27: 37-53.

Uddin, M., A. S. Chishti, S. Singh, U. H. Bhat, S. Singh and M. M. A. Khan. 2023. Effect of GA_3 and NAA on growth, physiological parameters, and bioactive constituents of *Ammimajus* L. Ind. Crop. Prod. 194: 116328.

Xanthopoulou, A., I. Ganopoulos, A. Kalivas, I. Nianiou-Obeidat, P. Ralli, T. Moysiadis, A. Tsaftaris and P. Madesis. 2015. Comparative analysis of genetic diversity in Greek Genebank collection of summer squash ('*Cucurbita pepo*') landraces using start codon targeted (SCoT) polymorphism and ISSR markers. Aust. J. Crop. Sci. 9: 14-21.

Xylia, P., A. Chrysargyris, D. Shahwar, Z. F. Ahmed and N. Tzortzakis. 2022. Application of rosemary and eucalyptus essential oils on the preservation of cucumber fruit. Horticulturae. 8(9): 774.

Yang, Q. Q., D. S. Zhao, C. Q. Zhang, H. Y. Wu, Q. F. Li, M. H. Gu, S.S. M. Sun and Q. Q. Liu. 2018. A connection between lysine and serotonin metabolism in rice endosperm. Plant Physiol. 176: 1965-1980.

Yang, Q., D. Zhao and Q. Liu. 2020. Connections between amino acid metabolisms in plants: Lysine as an example. Front. Plant Sci. 11: 928.

Yang, S. F. and N. E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol. 35: 155-189.

Yong, J. W. H., S. D. Letham, C. S. Wong and D. F. Graham. 2014. Rhizobium-induced elevation in xylem cytokinin delivery in pigeonpea induces changes in shoot development and leaf physiology. Funct. Plant Biol. 41: 1323-1335.

Zarrin, N., M. Shoor, A. Tehranifar and Z. Karimian. 2021. Effect of day length and gibberrelic acid on some morphological and biochemical characteristics of *Chrysanthemum.* Ir. J. Hortic. Sci. 51: 979-993.